Lower Motor Neuron Disease with Accumulation of Neurofilaments in a Cat

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Abstract. A young cat had signs of tetraparesis that progressed to tetraplegia within a few weeks. Clinically, there was lower motor neuron disease with areflexia and muscle atrophy in all limbs. Degeneration of the motor neurons in the spinal cord was seen on histological examination. Ultrastructurally, the degeneration of nerve cells was characterized by abnormal proliferation of neurofilaments. These findings were compared to other motor neuron diseases and neurofibrillary accumulations in man and animals.

Several diseases of the central nervous system in man are characterized by an abnormal accumulation of neurofibrillary material in neurons [16]. Ultrastructurally this material is either composed of fibrils resembling neurotubules such as in Alzheimer disease or of neurofilaments such as in sporadic motor neuron disease in children [11]. In animals accumulation of neurofilaments has been seen after experimental administration of aluminum compounds [15] and vinca alkaloids [6, 9]. Spontaneous diseases primarily characterized by neurofibrillary accumulation are rare in animals. We found only one recent report of such a disease in a dog [3].

Case History

A 10-week-old female, domestic, short-hair cat with tetraplegia was presented to the Auburn University Small Animal Clinic. At 3–4 weeks of age, weakness had been noted in all four limbs. The kitten trembled and collapsed while attempting to walk. The tetraparesis progressed to complete tetraplegia 3–4 weeks before the cat was admitted. The cat was the only one affected in a litter of four. The queen was clinically normal but no information could be obtained concerning the sire.
Materials and Methods

Neurological tests done were reflex evaluation, eye fundus examination, electroencephalography, electromyography and cerebrospinal fluid analysis.

The brain, spinal cord, segments of the brachial plexus, ischiadic and femoral nerves, muscles and representative samples of all extraneural tissues were removed at necropsy, fixed in 10% buffered formalin and processed for paraffin embedding. Whole coronal sections were cut at 4 μm at representative sections of the brain and spinal cord and stained with hematoxylin and eosin (HE), luxol fast blue-cresyl echt violet, Holmes' silver impregnation, and periodic acid-Schiff (PAS). Other tissue sections were stained with HE.

Parts of the ventral horns of the cervical and lumbar segments were dissected from the formalin fixed spinal cord. They were washed overnight in buffer solution and fixed in 1% osmium tetroxide for 1 h, dehydrated in graded ethanol and embedded in epon 812. Semi-thin sections were cut at about 1 μm and stained with toluidine blue for light microscopy. Silver to grey sections were cut from suitable blocks on an ultramicrotome and stained with uranyl acetate and lead citrate.

Results

On neurological examination the cat was mentally alert but lay immobile in sternal or lateral recumbency. Little voluntary movement of the limbs could be detected and weak attempts were made to hold the head erect. Postural reactions of wheelbarrowing, hopping, extensor postural thrust, hemiwalking and conscious proprioception were absent in all limbs. Muscle tone was reduced in all limbs and tail and on passive manipulation there was diffuse atrophy of the appendicular musculature. Segmental withdrawal and myotatic reflexes were absent. The perineal reflex was depressed and the anal sphincter slightly relaxed. Pain perception was in all four legs and tail despite a severe motor impairment. There was no evidence of abnormality of cranial nerves except for a slightly slow menace response. Electromyographic abnormalities were spontaneous fibrillation potentials in the appendicular muscles tested, including biceps and quadriceps femoris, triceps and biceps brachii. Normal insertion potentials with little spontaneous electrical activity were seen in the paraspinal muscles. Cell count, protein level, and cytomorphology of the cerebrospinal fluid were unremarkable. The animal was killed and submitted for postmortem examination.

Apart from marked atrophy of the pectoral and pelvic musculature, no gross lesions were found.

On tissue sections, widespread degeneration was seen in the large motor neurons of the lateral parts of the ventral horns in the cervical and lumbar intumescences of the spinal cord.
Fig. 1. Large ventral horn motor neuron. The Nissl substance is in irregular strands and clumps separated by spaces. Flattened nucleus is in periphery of the cell body. Luxol fast blue-cresyl echt violet.

Fig. 2. Large ventral horn motor neuron. Diffuse argentophilia with discrete whorl pattern. Holmes' silver.

The perikarya were swollen and nuclei were often displaced to the periphery. The Nissl substance was reduced to irregular strands and clumps separated by clear zones and in many cells the substance was almost completely missing (fig. 1). Karyorrhexis occurred in several degenerating neurons. In a few other cells, nuclear destruction accompanied by advanced chromatolysis resulted in the formation of ghost cells. A diffuse argentophilia with indistinct whorls was seen after silver impregnation of the affected neurons (fig. 2). After neurons were destroyed, they disappeared and glial nodules were formed. No lesions were found in the spinal cord, brain stem, cerebellum or cerebrum. There was Wallerian degeneration in the ventral spinal nerve roots and peripheral nerves of the affected segments. There were randomly distributed small angular fibers within muscle fiber groups from several of the appendicular muscles. Necrosis, infiltration with inflammatory cells or regeneration were not seen. The changes, therefore, were considered to be consistent with neurogenic atrophy. Typing of muscle fibers by histo-chemical means and histographic analysis, however, were not carried out.
Fig. 3. Part of the ventral horn motor neuron. Clumps of rough endoplasmatic reticulum (R) and mitochondria (M) are scattered among densely accumulated neurofilaments (NF). N = nucleus. Inset Neurofilaments with lateral projections. Uranyl acetate and lead citrate.

Large interlacing bundles of fibrillary material were conspicuous in the degenerated neurons (fig. 3). This material was composed of bundles of parallel fibrils with diameters of about 100 Å. A central core in the filaments could not be seen. Small lateral projections in a random distribution were obvious at higher magnification (fig. 3, inset). The neurofilaments also could be seen in some dendritic spines but not in axons. The normal arrangement of the rough endoplasmatic reticulum comprising the Nissl bodies was severely disturbed. Small fragments of rough endoplasmatic reticulum and numerous closely associated free ribosomes were scattered throughout the perikarya and appeared to be encroached upon by the surrounding fibrillary bundles (fig. 4). Associated with the disorganized Nissl fragments were large groups of extremely swollen mitochondria. The Golgi cisternae appeared flattened and numerous vesicular structures were often adjacent.
Fig. 4. Bundles of parallel neurofilaments (nf) cut longitudinally. Fragments of rough endoplasmatic reticulum (R) with free ribosomes. Uranyl acetate and lead citrate.

Discussion

Clinically, lower motor neuron diseases are difficult to characterize and differentiate without ancillary diagnostic and pathologic procedures. The clinical signs in this case were characterized by hypotonia and hyporeflexia primarily in the appendicular musculature; this correlates with the most severe degenerative changes in the neurons of the cervical and lumbar intumescences. In addition, the rapidly developing tetraplegia with severe muscle atrophy differentiates this disorder from other degenerative neuronal diseases in young cats.

The selective degeneration of the motor neurons justifies its classification among the so-called motor neuron diseases. This group includes amyotrophic lateral sclerosis and related conditions in man. In animals, motor neuron diseases are rare. A hereditary form of lower motor neuron and preganglionic sympathetic neuron degeneration was described in hybrid dogs resulting from breeding Bloodhounds or Great Danes with St. Bernards [13]. Pathologically 'Stockard's paralysis' was characterized by pronounced degeneration of the neurons in the ventral and intermediolateral horns of the lumbar spinal cord [13]. Lower motor neuron disease was reported in nine dogs of
various ages and breeds in which prominent loss of ventral horn motor neurons and degeneration of the peripheral motor nerve fibers occurred [5]. The hereditary neuronal abiotrophy in Swedish Lapland dogs is also considered to be a lower motor neuron disease [8]. Hereditary motor neuron degeneration with vacuolation of the nerve cells was reported in wobbler mice [1]. A recent publication described lower motor neuron degeneration in mice caused by a C-type RNA virus [4]. The lesions in our cat are different from any of the conditions mentioned above in that the neuronal degeneration was characterized by an abnormal proliferation of neurofilaments. Our case is similar, however, to all experimentally induced or spontaneously occurring neurofibrillary accumulations reported in animals, since the stored material is composed of neurofilaments [16]. The helically twisted pairs of filaments, previously thought to be neurotubules, have only been reported in human diseases [17]. Size and fine structure of the neurofilaments in our cat are similar to those described [3, 16]. There is, however, considerable variation among these diseases in the localization of the accumulated neurofilaments. Certain diseases evoke the change primarily in axons and others in the neuronal perikarya [16]. Fibrillary accumulations may also occur in cells other than neurons after administration of vinca alkaloids [6].

The selective topography of the lesions in the spinal cord is similar to sporadic motor neuron disease in which affected neurons are most numerous in the lumbar and cervical intumescences of the cord [10]. Others have reported that in the dog nerve cell lesions were found throughout the central nervous system without predilection for any particular area.

The cause of filamentous proliferation is not understood. One hypothesis is that the proliferation of neurofilaments after colchicine is administered occurs because structural proteins comprising the cytoplasmic microtubular structures depolymerize after specific binding of this drug with the structural protein [2, 12]. Transient or symptomatic accumulation of neurofilaments occurs as a reaction of the neuron to axonal injury [18]. In the cat, this was seen in the axonal terminals of Purkinje cells after experimental damage of the cerebellar cortex [7].

We wondered if the neuronal lesions in our cat were secondary to peripheral disease. We compared our findings to those in experimental studies on retrograde change [14], and found that both histological and ultrastructural lesions seem different. In our cat there was no central chromatolysis of the neuron, an occurrence in retrograde change. The chromatolytic pattern in the neurons of our cat was very irregular, mainly because of large deposits of argentophilic material in the neuronal perikarya. In retrograde change
there are fewer neurofilaments by far than there were in our cat; moreover, our cat lacked other organelle changes common in this condition [14].

In addition, no primary involvement of the peripheral nerves and the musculature was seen in our cat. The lesions in the spinal nerve roots and the peripheral nerves were consistent with Wallerian degeneration and those in the musculature with neurogenic atrophy.

The nature and selective localization of the lesions in the absence of other primary changes suggests a specific metabolic abnormality in our cat. The age of the cat and the very early onset of the clinical signs is also suggestive of a congenital disease. Breeding experiments are necessary, however, to establish the existence of an inborn error of neuronal metabolism and abnormal proliferation of neurofilaments.

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References